Electronic Supplementary Materials
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Physical interactions and mutational analysis of MoYpt7 in *Magnaporthe oryzae*

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Data S1 Materials and methods of mass spectrometry (MS)

After adjusting the pH to 8.5 with 1 mol/L ammonium bicarbonate, DTT to 10 mmol/L was added to the total protein (100 μg) extracted from each sample, chemical reduction reaction at 60 °C for 1 h and 45 min, and then carboxyamidomethylated with 55mmol/L iodoacetamide at room temperature for 45 min in dark. Then Trypsinase Gold (Promega, Madison, WI, USA) was added to the final substrate/enzyme ratio of 30:1 (w/w). The trypsinase digest was incubated at 37 °C for 16 h. After digestion, the peptide mixture was acidified with 10 μl of formic acid for further MS analysis. After digestion of the protein, each peptide sample was desalted using a Strata X column (Phenomenex) and dried in vacuum, and then resuspended in 200 μl of Buffer A 2% ACN, 0.1% FA). After centrifugation at 20000*g* for 10 min, the supernatant was recovered to give a peptide solution with a final concentration of about 0.5 μg/μl. 10 μl of the supernatant was loaded on LC-20AD nano HPLC (Shimadzu, Kyoto, Japan) through an autosampler into a C18 trapping column (diameter of 2 cm). The peptide sample was then eluted into an internal package of 10 cm C18 column (75 μm in inner diameter). Samples were loaded at 8 μl/min for 4 min and run 35 min with the flow of B from 2 to 35% B (95% ACN, 0.1% FA) at 300 nl/min, and then linearly gradient to 60% B for 5 min, followed by a linear gradient of 2 min to 80% B for 4 min, and finally to 5% B. The data acquisition was carried out using the TripleTOF 5600 system (AB SCIEX, Concord, ON) with Nanospray III source (AB SCIEX, Concord, ON) and the quartz tip as emitter (New Objectives, Woburn, MA). Data were obtained using a 2.5 kV ion spray voltage, a 30 psi curtain gas, a 15 psi nebulizer gas, and an interfacial heater temperature of 150 °C. For TOF MS scans, MS was run at RP operation greater than or equal to 30 000 FWHM. For IDA, a measurement scan is acquired within 100 ms and up to 40 product ion scans are collected if more than 150 (counts per second) and 2+ to 5+ charge states are exceeded. The total cycle time is fixed at 2.8 s. The Q2 transmission window is 80 Da and 100 Da is 50%. By detecting a 40 GHz multichannel TDC detector with four anode channels for detection of ions, each scan is summed for four time periods by a pulse generator frequency value of 11 kHz. Set the dynamic exclusion to 1/2 of the peak width (15 s), and then refresh the precursor from the exclusion list (BGI, China).